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REVIEW

Structure, function, and regulation of adrenergic receptors

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Abstract

Adrenergic receptors for adrenaline and noradrenaline belong to the large multigenic family of receptors coupled to GTP-binding proteins. Three pharmacologic types have been identified: α_1-, α_2-, and β-adrenergic receptors. Each of these has three subtypes, characterized by both structural and functional differences. The α_2 and β receptors are coupled negatively and positively, respectively, to adenylyl cyclase via G_i or G_s regulatory proteins, and the α_1 receptors modulate phospholipase C via the G_o protein. Subtype expression is regulated at the level of the gene, the mRNA, and the protein through various transcriptional and postsynthetic mechanisms. Adrenergic receptors constitute, after rhodopsin, one of the best studied models for the other receptors coupled to G proteins that are likely to display similar structural and functional properties.

Keywords: adrenergic receptors; G protein interaction domain; ligand binding site; regulation of subtype expression

The multiple metabolic and neuroendocrine effects of adrenaline and noradrenaline are mediated by a class of membrane-bound proteins designated as the adrenergic receptors (AR). The catecholamines activate various cellular signal transduction mechanisms by binding to these receptors, which in turn activate GTP-binding regulatory G proteins, themselves modulating effectors such as adenylyl cyclase or phospholipase C.

The adrenergic receptors are, after rhodopsin, the earliest and thus the most extensively studied group of G protein-coupled receptors, and are now known to constitute a very large family that includes receptors for many peptidic and nonpeptidic hormones, drugs, and neurotransmitters as well as for sensory stimuli such as light or olfactory substances. All of these receptors share important structural and functional properties: they are all composed of a single polypeptide chain with seven hydrophobic stretches likely to constitute seven transmembrane domains spanning the lipid bilayer, and they all are coupled to a GTP-binding protein.

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The properties of this "R_7G" family have been discussed in several recent reviews (Birnbaumer et al., 1990; Strosberg, 1991a). Here, I shall focus attention on the adrenergic receptors (Dohlman et al., 1991; Strosberg, 1991b) and shall discuss the current status of the research and its future directions.

Members of the adrenergic receptor family

Nine subtypes

Adrenaline and noradrenaline act on a large variety of tissues by binding to α- and β-adrenergic receptors (Ahlquist, 1948). A more detailed analysis led Lands (1967) to pharmacologically distinguish α_1 and α_2 and β_1 and β_2 subtypes. Cloning and sequencing of the corresponding mostly intronless genes and pharmacologic analysis of their products expressed in transfected cells have resulted in the definition of three types: α_1, α_2, and β, with the identification for each of them of three subtypes: α_{1A}, α_{1B}, and α_{1C}; α_{2A}, α_{2B}, and α_{2C}; and β_1, β_2, and β_3. Although not all the subtypes have been studied as extensively as the β_2 receptor, which is discussed at length below, quite a large amount of information has now be-

come available about the structure and function of all the adrenergic receptors.

Primary structures

The primary structures deduced from the nucleotide sequences of the nine adrenergic receptor subtypes are compared in Figure 1 and clearly demonstrate that all these subtypes display similar characteristic features: a single polypeptide chain from 400 to over 500 residues long comprising amino- and carboxy-terminal regions variable both in length and in sequence, and three intracellular ("i"), three extracellular ("e"), and seven well-conserved hydrophobic, possibly transmembrane ("tm"), stretches. The α_2 receptor subtype C-terminal regions are shorter than those of the β and much shorter than those of the α_1 subtypes, in line with the observation that receptors involved in the stimulation (e.g., βAR) or inhibition (e.g., α_2AR) of adenylyl cyclase generally have short i3 and C-terminal segments, whereas receptors involved in other effector systems such as phospholipase C (α_1AR) have longer sequences in these regions. The human α_{2B} thus has a 23-residue C-terminus, whereas the human α1B C-terminal region is 167 residues long (Fig. 1).

A detailed description of all available information on adrenergic receptors would clearly go beyond the scope of this article and can be found in a number of recent reviews (Harrison et al., 1991; Strosberg, 1991b; Bylund, 1992; Kobilka, 1992; Ostrowski et al., 1992), but I have attempted to summarize in Table 1 some of the salient features of the human adrenergic receptors and will discuss below further molecular characteristics of the ligand-binding and G protein-coupling domains. In this table, I present pharmacologic properties in terms of agonists and agonists reported to bind to or stimulate one subtype better than any other subtype with the caveat that no single drug suffices to define a given receptor. I also indicate which effector mechanism is triggered best, remembering, however, that secondary effectors may sometimes also be activated.

Adrenergic receptors: A family portrait

Because of their scarcity, affinity chromatography of detergent-solubilized adrenergic receptors was the method of choice to purify the first adrenergic receptors to be studied: the β_1-like turkey erythrocyte (Vauquelin et al., 1977, 1979b) and the β_2 hamster lung receptors (Caron et al., 1981). Partial amino acid sequencing of a few tryptic peptides led to the synthesis of oligonucleotides that were used as probes to clone the corresponding hamster β_2 cDNA (Dixon et al., 1986), turkey β_1-like cDNA (Yarden et al., 1986), and the human platelet α_{2A} cDNA (Kobilka et al., 1987).

Hydropathy plots of the predicted amino acid sequences revealed the presence of the seven hydrophobic segments, previously identified as transmembrane domains in bacteriorhodopsin. Despite the lack of amino acid sequence homology, the similarity with the bacterial light receptor led to a series of fruitful studies that basically sustained the hypothesis that all seven hydrophobic segments contribute to form a ligand-binding pocket, as was recently demonstrated for bacteriorhodopsin by high-resolution electron cryoscopy (Henderson et al., 1990) and by biochemical (Dohlman et al., 1991) and immunologic techniques (Wang et al., 1989) for the β-adrenergic receptors.

I present in Figure 2 the membrane topography of a typical G protein-coupled mammalian receptor, the human β_2 receptor, the gene of which was cloned by Emorine et al. (1987) and Kobilka et al. (1987) based on its homology with hamster cDNA (Dixon et al., 1986).

The human protein is composed of a single polypeptide chain of 413 amino acid residues with an extracellular N-terminus containing two consensus sites for N-linked glycosylation (see Figs. 1, 2), seven transmembrane segments of 21–28 residues, three extra- and three intracellular loops, and an intracellular C-terminus containing two sites for phosphorylation by protein kinase A as well as several sites for phosphorylation by the β-adrenergic receptor kinase.

Postsynthetic modifications

N-linked glycosylation

All adrenergic receptor subtypes, except the α_{2B} of rat and man, display one, or more often two, Asn-X-Ser/Thr consensus sites for *N*-glycosylation in the amino-terminal region (Fig. 1). *N*-linked carbohydrates may account for as much as a quarter of the apparent weight of the adrenergic receptor proteins. Lack of polysaccharide addition (as is the case for βAR receptors functionally expressed in *Escherichia coli* [Marullo et al., 1989; Strosberg & Marullo, 1992]), partial or complete inhibition of glycosylation by monensin or tunicamycin, or removal by specific enzymes (reviewed in Ostrowski et al. [1992]) does not seem to alter ligand binding or signal transmission in any G protein-coupled receptor yet examined. Absence of carbohydrates does appear, however, to reduce considerably the density of β_2AR expressed at the surface of A431 cells (Cervantes et al., 1985, 1988). Carbohydrates may thus play a role in receptor trafficking.

Palmitoylation

All adrenergic receptor subtypes except the α_{2C} display a Cys residue immediately after the tm7 domain. In the β_2AR, palmitoylation of this residue, situated at position 341, has been shown to contribute significantly to the ability of the agonist-bound receptor to mediate adenylyl cyclase stimulation, possibly by promoting the insertion of several adjacent residues in the membrane (O'Dowd et al.,


e1
tm1
i1
tm2
e2
tm3
Topology
β1
β2
β3
α1A
α1B
α1C bovine
α2A
α2B
α2C
i2
tm4
e3
tm5
i3
tm6
e4
tm7
i4
i4

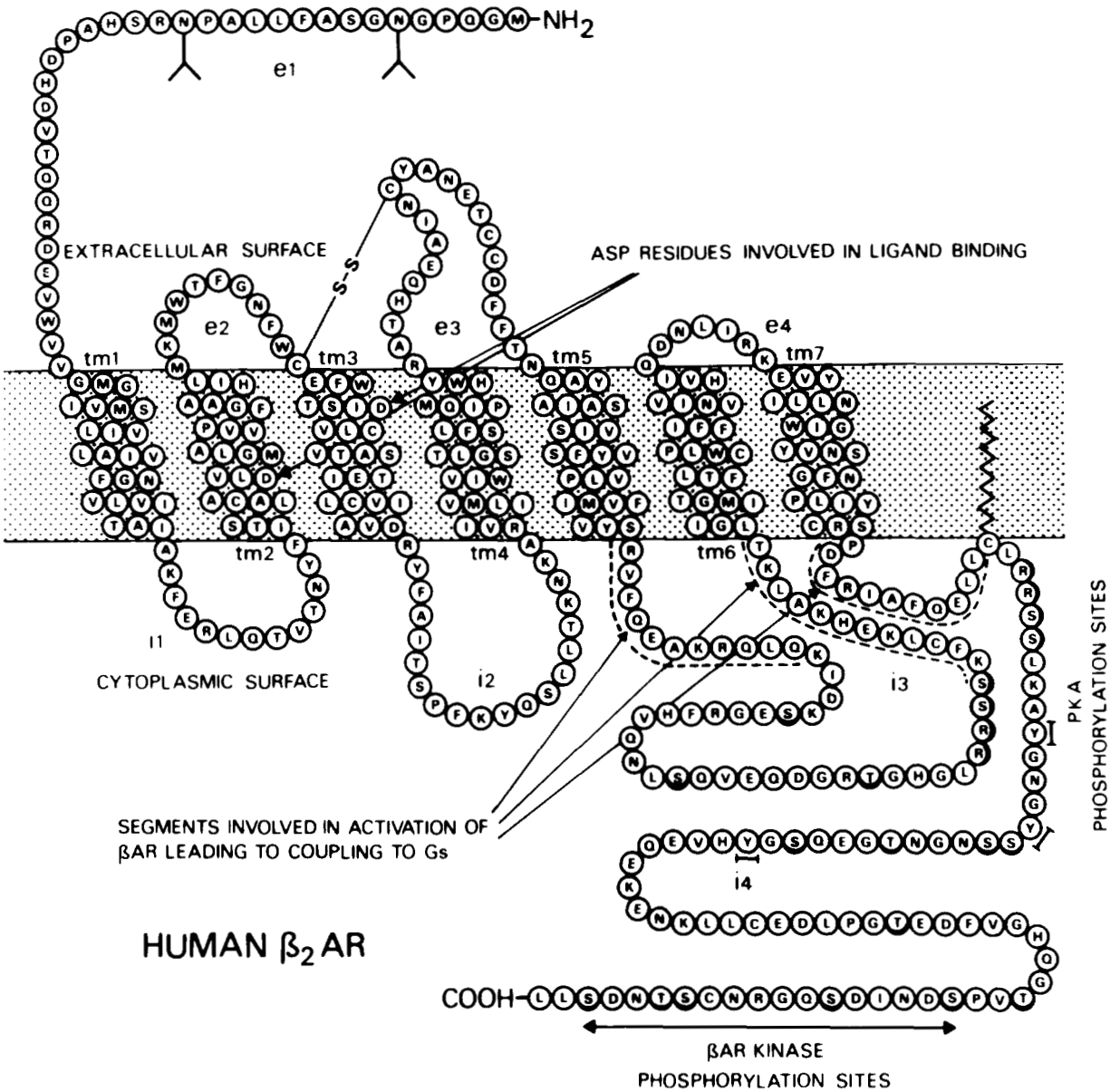

Fig. 2. Prototypic model of the human β_2-adrenergic receptor. The single polypeptide chain is arranged according to the model for rhodopsin. The disulfide bond, essential for activity, linking Cys^{106} and Cys^{184} is represented by -S-S-. The two *N*-glycosylation sites in the amino-terminal portion of the protein are indicated by λ. The palmitoylated Cys^{341} residue in the N-terminus of the i4 loop is indicated by ∧∧. Potential Ser and Thr phosphorylation sites are underlined. The three Tyr residues found in the i4 of β_2-, but not in β_1- or β_3AR, are indicated by ⊢⊣ (modified from Kobilka et al. [1987]).

1989; Moffett et al., 1993) and thus forming a fourth intracytoplasmic loop resulting in an active conformation for G protein coupling. Lack of palmitoylation has been associated with constitutively increased phosphorylation of the β_2AR, suggesting that the absence of this fourth loop renders phosphorylation sites accessible to regulatory mechanisms (Moffett et al., 1993). The recently suggested agonist modulation of receptor palmitoylation may actually constitute itself yet another control mechanism (Mouillac et al., 1992).

Fig. 1 (*facing page*). Amino acid sequences of the adrenergic receptor subtypes derived from the nucleotide sequences of the cloned cDNA and genes. The seven transmembrane segments (tm1-tm7) alternate with extracellular (e1-e4) and intracellular (i1-i4) domains. Gaps have been introduced to maximize homology. References: β_1 (Emorine et al., 1987; Frielle et al., 1987); β_2 (Emorine et al., 1987; Kobilka et al., 1987); β_3 (Emorine et al., 1989); α_{1A} (Bruno et al., 1991); α_{1B} (Ramarao et al., 1992); α_{1C} (Schwinn et al., 1990); α_{2A} (Guyer et al., 1990); α_{2B} (Lomasney et al., 1990); α_{2C} (Regan et al., 1988).

Table 1. *The human adrenergic receptors*[a]

	β			α_1[b]			α_2[b]		
	$\beta 1$	$\beta 2$	$\beta 3$	α_{1A}	α_{1B}	α_{1C} (bovine)	α_{2A}	α_{2B}	α_{2C}
Pharmacology									
Potent agonist	Iso > bucindolol, norepi	Clenbuterol, epi ≥ iso	Isoproterenol > norepi, bucindolol	Phenylephrine	Phenylephrine	Phenylephrine	Norepi, epi	Norepi, epi	Norepi, epi
Selective agonist	Xamoterol	Procaterol	CL316,243				Oxymetazoline		
Selective antagonist	CGP20712A	ICI 118551	Bupranolol[c]	5-Methyl urapidil, Prazosin	Prazosin	Prazosin		ARC 239(100)	Prazosin
Mechanism of action	↑cAMP	↑cAMP	↑cAMP	Ca^{2+} channel	IP_3/DG	IP_3/DG	↓cAMP, K^+ channel↑, Ca^{2+} channel↓	↓cAMP, Ca^{2+} channel↓	↓cAMP
Gene									
Chromosome no.	10	5	8	5	5	8	10	2	4
Existence of introns	No	No	Yes		Yes	Yes	No	No	
Protein structure									
Length	477	413	408	515	517	466	450	451	461
Glycosylation sites (N-terminal)	2	2	2	2	4	3	2	0	2
Phosphorylation sites	PKA, βARK	PKA, βARK	0		PKA	PKA	PKA, βARK		
Prototypic tissue	Heart	Lung	Fat	Artery (renal; rat)	Heart		Platelet, brain		Spleen

[a] epi, epinephrine; norepi, norepinephrine; IP_3, phosphatidyl inositol triphosphate; DG, diacyl glycerol; PKA, protein kinase A; βARK, β-adrenergic receptor kinase (sites predicted on the basis of consensus target sequences). Blanks indicate that no definitive conclusion can be proposed.

[b] Previous reports mention the existence of additional α_1 (α_{1D}) and α_2 (α_{2D}) subtypes.

[c] Bupranolol is the best β_3 antagonist identified so far, but it is not selective for this subtype.

Disulfide bond formation

The treatment by reducing agents of the turkey β_1-like AR leads to loss of ligand binding, which can be prevented by the presence of agonists or antagonists (Vauquelin et al., 1979a). At least one disulfide bond, probably formed between Cys^{106} and Cys^{184} in the β_2AR, has also been suggested to be essential for ligand binding by site-directed mutagenesis (Dixon et al., 1987) and functional studies (Dohlman et al., 1990) in hamster and human β_2AR. Cys residues in positions homologous to those of the βAR are found in nearly all receptors coupled to G proteins, and the bond formed between them may thus constitute an additional conserved feature of the R_7G family of proteins. A second disulfide bond may form between the Cys^{190} and Cys^{191}, which are only present in the three βAR.

Ligand binding and signal transmission in adrenergic receptors

The ligand-binding pocket

The adrenergic ligand-binding site is formed by the seven membrane-spanning domains and may be represented as seen in Figure 3; removal of most of the amino- or carboxy-terminal residues by proteolysis (Rubenstein et al., 1987; Wong et al., 1988) or by deletion mutations of β_2AR (Dixon et al., 1987) or α_2AR (Wilson et al., 1990) has little or no effect on the binding of ligands to the adrenergic receptor. Fluorescence quenching analysis indicated that the β_2 antagonist carazolol is in fact buried at least 10.9 Å deep into the hydrophobic core of the receptor (Tota & Strader, 1990).

Photoaffinity labeling and site-directed mutagenesis studies have helped in the identification of receptor residues belonging to regions in close proximity to the ligand. Two types of residues have thus been identified: those associated with agonist binding and those involved in G protein activation. A number of such residues are represented in Figure 4, which shows key interactions between the β_2AR and the agonist noradrenaline.

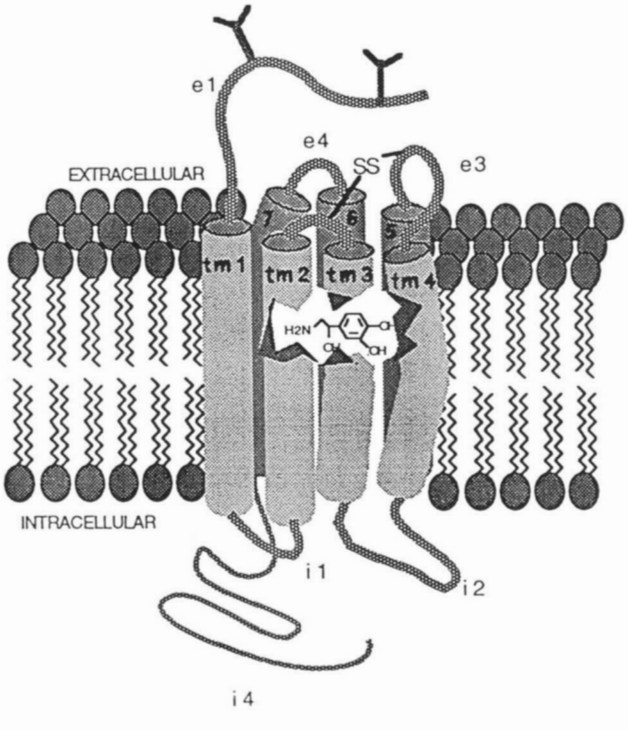

Fig. 3. Topological model of the β_2-adrenergic receptor inserted in the membrane and of the ligand-binding pocket. The ligand-binding region formed by seven transmembrane domains is buried in the lipidic bilayer (Strosberg, 1991b).

Ligand-binding residues

The most important residue is undoubtedly Asp^{113} in tm3, which is conserved in all adrenergic receptors, indeed in all monoamine receptors analyzed so far. Its carboxylate group is believed to act as a counter-ion for the amino group present in the ligand; substitution of this aspartate by any other residue except glutamate abolishes binding of monoamines (Dixon et al., 1988). When a β_2AR mutant was generated in which glutamate replaced aspartate, the β_2 antagonists pindolol and oxprenolol were then recognized as partial agonists (Strader et al., 1989b). Position 113 is clearly crucial for receptor function; when the aspartate is changed to serine, catechol esters that may form a hydrogen bond with the hydroxyl group of this residues become able to act as agonists toward the mutant receptor (Strader et al., 1991).

The role of hydrogen bonds is also important in other positions; site-directed mutagenesis suggests that Ser^{204} (tm5) forms a hydrogen bond with the meta-hydroxyl group of the catechol ring and that Ser^{207} (also in tm5) forms a hydrogen bond with the para-hydroxyl group (Strader et al., 1989a).

Mutation of several other residues in different transmembrane domains also affects ligand binding (Strader et al., 1987a; Dixon et al., 1988), including Ser^{165} (tm4), Phe^{290} (tm6), and the four cysteine residues Cys^{106}, Cys^{184}, Cys^{190}, and Cys^{191} mentioned before. Whereas most of these observations were done on β_2AR, Link et al. (1992) showed that substitution of yet another residue, Cys^{201}, by Ser (tm5) in human α_{2A}AR generated the antagonist binding properties of its murine counterpart.

Signal transmission

While Asp^{113}, Ser^{204}, and Ser^{207}, which exist in homologous positions in all adrenergic receptors, appear essential for ligand binding, other residues undoubtedly participate both in binding and in signal transmission, leading to activation of the α subunit of the G protein

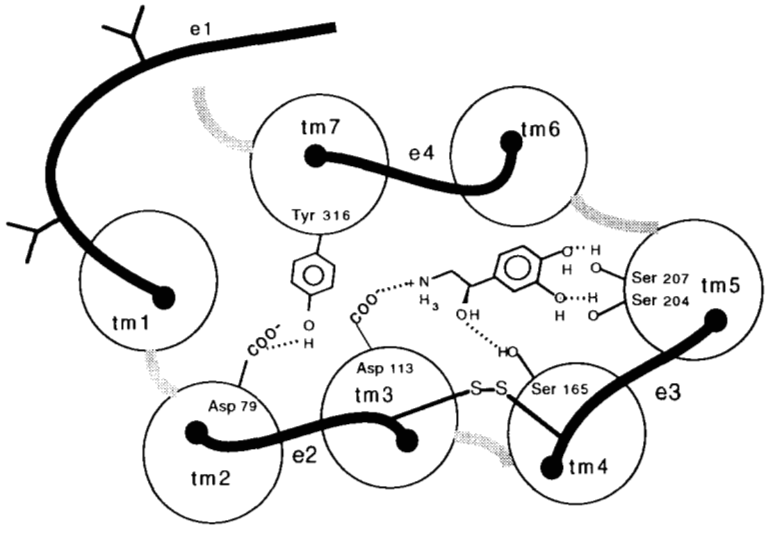

Fig. 4. Schematic view of the chemical interactions of noradrenaline with various residues of the β_2-adrenergic receptor binding site. Composite image of the β_2AR ligand-binding region. Proposed interactions in the ligand-binding area of the βAR viewed from the outside of the cell. All seven tm domains are essential for ligand binding. The ligand noradrenaline is shown surrounded by several of the amino acid chains that are speculated to be involved in agonist binding. These are Asp^{113} in tm3, Ser^{165} in tm4, and Ser^{204} and Ser^{207} in tm5. The movement of Tyr^{316} after agonist binding toward Asp^{79} may be important for signal transmission to G_s. Whether all the interactions with the ligand occur simultaneously or sequentially is not known (Strosberg et al., 1993).

coupled to the receptor. This is the case for Asp^{79} (tm2); substitution of this residue in the β_2 receptor results in severe loss in affinity for agonists, whereas antagonist binding remains essentially unchanged (Strader et al., 1987; Breyer et al., 1990).

In the α_{2A} receptor, substitution of Asp^{79} by Asn resulted in the loss of coupling to potassium current modulation without affecting agonist-induced adenylyl cyclase or voltage-dependent calcium current inhibition (Surprenant et al., 1992). Because distinct G proteins appear to couple adrenergic receptors to potassium (G_i) or to calcium (G_o) currents, these authors concluded from their study that the single point mutation affected only the ability of the receptor to activate the G_i protein that mediates potassium channel responses.

Three other residues in β_2AR appear to play a major role in signal transmission. Substitution of Asp^{130} by Asn results in a receptor with high-affinity agonist binding that is uncoupled from adenylyl cylase (Fraser et al., 1988). Two other crucial residues are Tyr^{316} and Asn^{312} (both in tm7). A multistep dynamic model has been proposed (Strosberg et al., 1993) in which the agonist-induced interaction of Asp^{79} with Tyr^{316} may constitute the first step toward activation of the G protein. Antagonist binding would promote formation of a hydrogen bond between Tyr^{316} and Asn^{312} and thus prevent G_s activation.

Determinants of subtype-selective binding/signal transmission

Results from α-β and β_1-β_2 chimeric receptor studies have confirmed that determinants of subtype selectivity are found on several if not all of the seven transmembrane domains. Replacement of the entire tm5-i3-tm6 region of α_{2A}AR by the corresponding region of β_2AR resulted in a chimeric receptor capable of stimulating adenylyl cyclase in response to α_2 agonists, albeit with reduced efficiency (Kobilka et al., 1988). A similar chimeric α_1/β_2AR was able to stimulate both phospholipase C (the α_1 effector) and adenylyl cyclase (Cotecchia et al., 1992).

The seventh domain appears to be important in determining differences in antagonist binding specificity between β_2AR and α_2AR (Kobilka et al., 1988). Substitution of the Phe^{412} by Asn in the human platelet α_2AR, for example, led to the loss of binding of the α_2 antagonist yohimbine and the acquisition of high affinity for the β_1/β_2 antagonists alprenolol, propranolol, and pindolol but not sotalol (Suryanaryana et al., 1992). In contrast, Wilson et al. (1990) reported that a proteolytic product of porcine α_2AR that contained only tm1-tm5 is capable of binding antagonists on its own.

Studies of β_1/β_2 chimeric receptors, on the other hand, confirmed that all the tm domains appear to contribute residues forming the ligand binding site (Dixon et al., 1988; Frielle et al., 1988). These authors suggested a progressive change in relative potency of the β_1-selective antagonist betaxolol and the β_2-selective antagonist ICI-118551 when domains of β_1 were replaced by domains of β_2, but a more detailed analysis of an extensive number of chimeric β_1-β_2 receptors functionally expressed in *E. coli* (Marullo et al., 1990) demonstrated that in fact each of 11 selective ligands appears to define its own ligand binding subsite.

Site of interaction with G proteins

Residues composing the amino- and carboxy-terminal segments of the third intracellular loop (i3) appear to constitute the main site of interaction with G proteins. This was demonstrated by studying the effects of deletion and homologous replacements by sequences from other receptors. In one such study, Wong et al. (1990) exchanged a 12-amino acid sequence in the N-terminus of i3 of the muscarinic M1 receptor with the homologous stretch

from turkey βAR. Binding of the muscarinic agonist acetylcholine to the M1-β chimera led to activation of both phospholipase C (the M1 effector) and adenylyl cyclase (the β effector). Replacement of the i2 M1 sequence by the turkey βAR sequence in the chimera conserved stimulation of cyclase but reduced activation of phospholipase C.

In fact, a single peptide corresponding to the C-terminus of i3 of human β_2AR is sufficient to activate the G_s protein (Okamoto et al., 1991). When this peptide is phosphorylated, as is the case of the whole β_2AR during the process of desensitization (see Regulation by postsynthetic modifications, below), the peptide loses its ability to stimulate G_s but displays an increased ability to stimulate G_i.

Substitution of just three residues from the carboxy-terminus of i3 of the α_{1B}AR by the homologous β_2AR amino acids ($Arg^{288} \rightarrow$ Lys, $Lys^{290} \rightarrow$ His, and $Ala^{293} \rightarrow$ Leu) resulted in an increase in both the binding affinity of norepinephrine and its potency to stimulate phospholipase C-mediated phospho-inositol turnover by two to three orders of magnitude and rendered the receptor constitutively active in the absence of agonist-induced activation (Cotecchia et al., 1990). More recently, the same group showed (Allen et al., 1991) that the α_{1B}AR gene, when overexpressed and activated by agonist, may function as an oncogene inducing neoplastic transformation. The mutational alteration of this gene may actually result in the constitutive activation of the protooncogene. Reciprocal mutation in the β_2AR also led to a constitutively activated receptor (Samama et al., 1993).

The comparison of the three βAR (Fig. 1) reveals that most of the residues believed to be responsible for G protein interaction, including those in i4 proximal to the membrane up to Cys^{341}, are well conserved, in line with the finding that all three subtypes interact with the same α_s subunit of G_s protein.

Regulation of subtype expression

The coexistence, even in the same cells, of several subtypes of receptors that may bind the same natural agonists, albeit with varying affinities, suggests an important role for regulatory mechanisms acting at the level of the gene or the protein.

Developmental regulation

Genes encoding the various adrenergic receptor subtypes may each respond to different signals during ontogenesis; the majority of β_3-specific mRNA is thus detected in brown adipose tissue, which, in mammals other than rodents, is found mainly in newborns or in rare pathological situations such as pheochromocytoma (Krief et al., 1993). Isolated brown or white adipocytes found throughout the life span of human adults do, however, express this β_3AR at the same time as they express β_1AR and β_2AR (Lönnqvist et al., 1992; Krief et al., 1993). In rodents, the β_3 subtype is the predominant subtype expressed in brown adipose tissue. In the murine 3T3-F44-2A fibroblasts only β_1 and β_2 are detected, but when induced to differentiate into adipocytes, these cells start to express predominantly β_3AR, and β_2AR becomes barely detectable (Fève et al., 1991).

Pharmacological regulation

In the same 3T3-F44-2A adipocyte-like cells, the expression of the β_2AR may be considerably up-regulated, and that of the β_1AR and β_3AR almost completely suppressed by treatment with the glucocorticoid dexamethasone (Fève et al., 1992). This up-regulation of β_2AR, previously described in transfected cell types (Emorine et al., 1987; Collins et al., 1991), may be explained by the existence in the 5′ flanking region of the β_2AR of several glucocorticoid-responsive element (GRE) consensus sequences that are potential sites of interaction with the glucocorticoid receptor (Emorine et al., 1991). Although analogous sequences may also be recognized in the β_3 5′ region, they are situated close to AP-1 binding sites; in other genes, such a proximity resulted in negative regulation by dexamethasone (Diamond et al., 1990).

Cyclic AMP-responsive elements (CRE) have also been identified in the 5′ flanking region of βAR. In β_2AR, Collins et al. (1990) showed at least one CRE. In β_3AR, three CRE seem effectively to regulate agonist-induced increased transcription of the receptor gene by cAMP (Thomas et al., 1992).

Regulation by postsynthetic modifications

Phosphorylation by protein kinase A

All adrenergic receptor subtypes, except β_3AR, contain at least one and sometimes two consensus target sites for phosphorylation by protein kinase A (PKA). These Arg/Lys-Arg-X-(X)-Ser/Thr sequences all occur in the i3 or carboxy-terminal domains, close to the sites of G protein interaction (Fig. 1). Absence of these sites, as in β_3AR (Emorine et al., 1989; Nantel et al., 1993), or their removal by mutagenesis (Hausdorff et al., 1989) almost completely prevents desensitization by low (nanomolar) concentrations of agonist. In a β_2-β_3AR chimera, the reintroduction of PKA and βARK phosphorylation sites partially restored rapid agonist promoted desensitization (Nantel et al., 1993).

In β_1AR and β_2AR, PKA phosphorylation promoted by agonist leads to down-regulation of receptor mRNA levels (Bouvier et al., 1989; Hadcock et al., 1989). In agonist-treated DDT1-MF2 vas deferens smooth muscle cells, decrease of β_1AR and β_2AR levels has recently be proposed to be preceded by binding to the corresponding mRNA of a 35-kDa protein that does not bind, in the

same cells, to α_{1B} mRNA, a subtype that does not undergo agonist-induced down-regulation (Port et al., 1992).

Phosphorylation by β-adrenergic receptor kinase

Phosphorylation of agonist occupied receptors may also occur in mutant β_2AR lacking the PKA sites (Hausdorff et al., 1989) or in the presence of PKA inhibitors (Lohse et al., 1989), leading again to receptor desensitization, but only in relatively high agonist concentrations (micromolar). This type of phosphorylation is caused by an enzyme that is functionally related to rhodopsin kinase and has been named β-adrenergic receptor kinase (βARK) because it was initially thought to act only on agonist-loaded βAR (Benovic et al., 1987), although it has now been shown to phosphorylate several other types of G protein-coupled receptors including α_{2A}, muscarinic M2, and even rhodopsin.

In contrast to PKA, βARK action does not directly interfere with activation of G_s. However, rhodopsin kinase mediates desensitization of rhodopsin by causing binding of arrestin to the phosphorylated protein, thus disrupting the interaction with transducin, the G protein involved in light adaptation. By analogy, Lohse et al. (1990) identified a β-arrestin that appeared to interfere with G_s activation of adenylyl cyclase.

The Ser/Thr target sites for βARK are most likely located in the carboxy-terminal region of the β_2AR; replacement or deletion of all of the 11 Ser and Thr residues closest to the C-terminus resulted in marked attenuation of agonist-stimulated rapid phosphorylation and receptor desensitization (Bouvier et al., 1988). The human α_{2A}AR lacks Ser or Thr residues in its very short C-terminus, but does contain such amino acids in i3, which thus probably constitutes the target site of βARK in this subtype (Liggett et al., 1992).

Phosphorylation by tyrosine kinase

The β_2AR also contains in its C-terminus a consensus site for phosphorylation by a tyrosine kinase. Treatment of hamster vas deferens smooth muscle cells with insulin promotes a marked desensitization of βAR-mediated activation of adenylyl cyclase. Phosphoamino acid analysis of immunoprecipitated β_2AR revealed increased phosphorylation of tyrosine and decreased phosphorylation of threonine residues (Hadcock et al., 1992). Although these authors did not attempt to identify the precise residue modified by the tyrosine kinase, it could possibly be Tyr^{366}, which is the only one belonging to a consensus site for that enzyme (Strosberg, 1991b; Hadcock et al., 1992). While phosphorylation has not been shown to contribute to down-regulation of the receptor, two other tyrosine residues—Tyr^{350} and Tyr^{354}—have been proposed to play an important role in this phenomenon (Valiquette et al., 1990).

Additional subtypes

The rapid expansion of the number of adrenergic receptor subtypes identified by molecular cloning has led scientists to wonder how many more would be found. A lucid analysis of the literature has in fact matched most of the known pharmacologic data with the properties of the cloned receptors expressed in model systems, suggesting that most adrenergic receptor subtypes have now been cloned. Remaining discrepancies have been linked to species differences rather than to evidence for more receptor subtypes; for example, reports that the human β_3AR is pharmacologically different from the rat β_3AR receptor and may therefore represent yet another receptor subtype have not been borne out when sufficient numbers of ligands were tested in several homologous well-controlled testing systems. Single residue differences between rat and human α receptors explain variations in pharmacologic properties, proposed by some investigators to reflect the existence of different subtypes. Finally, the ability of α-specific compounds to interact also with nonadrenergic imidazoline receptors may also explain apparent discrepancies in receptor properties.

Future research

The molecular characterization of three receptor subtypes for each type of adrenergic receptors (α_1, α_2, and β) now provides ample opportunities for future research. The comparison of homologous proteins that bind the same natural agonists with comparable affinities but synthetic agonists or antagonists with widely varying affinities will allow exquisitely precise structure–activity relationship studies. Site-directed mutagenesis, affinity labeling analysis, and ultimately X-ray diffraction of the crystallized proteins should help establish a well-defined picture of the ligand binding site and contribute to elucidate changes in conformation leading to signal transmission and G protein activation.

By completing these studies with the definition of the pharmacophores adapted to each subtype, selective drugs will become available to serve as therapeutic agents. This, however, will require the accurate definition of the physiologic function of each subtype, which will first depend on the exact tissue localization by in situ hybridization or by antibody detection. The striking evidence for differential subtype regulation at the protein and mRNA levels also offers new avenues for future therapeutic approaches that act on gene expression rather than on receptor function itself.

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